

WHAT IS CLAIMED IS:

1. A method for determining the ambient concentration of an analyte of interest among a plurality of analytes in a liquid sample of volume V
5 litres, comprising:

loading a plurality of different binding agents, each being labelled with a marker and being capable of reversibly binding an analyte which is or may be present in the liquid sample and is
10 specific for said analyte as compared to the other components of the liquid sample, onto a support means at a plurality of spaced apart small spots such that not more than 0.1 V/K moles of binding agent are present on any spot, where K litres/mole is the
15 affinity constant of said binding agent for said analyte;

contacting the loaded support means with the liquid sample to be analyzed, such that each of the spots is contacted in the same step with said
20 liquid sample, the amount of liquid used in said sample being such that only an insignificant proportion of any analyte present in said liquid sample becomes bound to said binding agent specific for said analyte;

25 contacting the support with a site-recognition reagent specific for each binding agent in a competitive or non-competitive technique, the site-recognition reagent being capable of recognizing either the unfilled binding sites or the filled
30 binding sites on said binding agent, said site-recognition reagent being labelled with a marker different from the marker on said binding agent, and
measuring a ratio of signals from said markers on the site recognition reagent and the
35 binding reagent from at least a part of the spot, from which the analyte to interest is determined.

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2. A method according to claim 1, wherein the markers on the site-recognition reagent and the binding reagent are fluorescent markers.

3. A method according to claim 2, wherein
5 the ratio of signals is measured using a laser scanning confocal microscope.

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